EEDP-01-7
March 1987

Environmental Effects of Dredging Technical Notes

USE OF *DAPHNIA MAGNA* TO PREDICT CONSEQUENCES OF BIOACCUMULATION

PURPOSE: Results reported herein represent a portion of the laboratory research evaluating the relationship between mercury and cadmium tissue residues and biological effects in the freshwater crustacean, *Daphnia magna* (commonly known as the water flea). Procedures presented here for a 28-day *Daphnia magna* toxicity test could be used in screening for water-column toxicity resulting from open-water disposal of a specific dredged material.

BACKGROUND: As a part of its regulatory and dredging programs, the U. S. Army Corps of Engineers often conducts, or requires to be conducted, an assessment of the potential for bioaccumulation of environmental contaminants from sediment scheduled for dredging and open-water disposal. There is, at present, no generally accepted guidance available to aid in the interpretation of the biological consequences of bioaccumulation. To provide an initial basis for such guidance, the Environmental Laboratory is conducting both literature database analyses and experimental laboratory studies as part of the Long-Term Effects of Dredging Operations (LEDO) Program.

ADDITIONAL INFORMATION OR QUESTIONS: Contact one of the authors, Dr. Thomas Dillon (601) 634-3922 (FTS 542-3922) or Ms. Alfreda Gibson (601) 634-4027 (FTS 542-4027), or the manager of the EEDP, Dr. Robert M. Engler (601) 634-3624 (FTS 542-3624).

Materials and Methods

Laboratory cultures of *Daphnia magna* were maintained in 1-ℓ culture dishes (eight adult *Daphnia* per dish) set in a constant temperature water bath at 20.0° C with a 14-hr photoperiod. Reconstituted water with a hardness of 180 mg/ℓ, as $CaCO_3$, and a pH of 8.2 was used in the culture medium (Dunbar et al. 1983). The *Daphnia* were fed every day (except Sunday) from a laboratory culture of the green algae, *Ankistrodesmus falcatus*, at a ration equivalent to 1.71 mg of dry algae for each container.

US Army Engineer Waterways Experiment Station, Environmental Laboratory
PO Box 631, Vicksburg, Mississippi 39180-0631

Exposure to mercury and cadmium began with <24-hr-old neonates having a mean dry weight of 21.0 µg and a mean length of 1.58 mm. The *Daphnia* were exposed to 0.0, 0.05, 0.1, 0.5 and 1.0 µg/ℓ mercury and, separately, to 0.0, 0.1, 0.5, 1.0 and 5.0 µg/ℓ cadmium under static renewal conditions. There were 12 replicate beakers per concentration and each contained 200 ml of water and 2 *Daphnia*. Each beaker was covered with a black petri dish. Reconstituted hard water was used throughout the test, and temperature, photoperiod, and feeding ration were identical to that used to maintain the laboratory *Daphnia* culture.

All beakers were checked daily for mortality, neonate production, and any abnormal behavior. Mortality was defined as cessation of all visible signs of movement of the second antennae, respiratory appendages, and the postabdomen after 5 sec of observation (Buikema et al. 1976). When discovered, neonates and dead adults were removed, counted, and discarded. Test solutions in all beakers were renewed each Monday, Wednesday, and Friday. Microliter volumes of mercury or cadmium were added from stock solutions prepared with mercuric chloride and cadmium chloride, respectively, dissolved in reverse osmosis (R.O.) water.

At the termination of the test, the *Daphnia* were rinsed three times in R.O. water. Lengths were determined by measuring from the top of the head to the base of the caudal spine using a dissection scope equipped with an ocular micrometer. The *Daphnia* were then individually placed in preweighed aluminum foil pans and dried for 24 hr at 70° C. After cooling in a desiccator for 2 hr, the *Daphnia* were weighed and dry weights were obtained to the nearest 1.0 µg. Eight daphnids were pooled per sample (three samples per treatment) in 20-ml vials containing 1.0 ml of 50-percent nitric acid, HNO_3. After digestion at 70° C for 24 hr, volumes were adjusted to 6.0 ml with R.O. water and analyzed for total mercury via atomic absorption after gold amalgamation formation. Cadmium tissue and water samples were analyzed via atomic absorption spectroscopy. Water samples were collected immediately after one renewal period during the experiment. Water from four replicate beakers was combined to yield one pooled water sample of 125-ml volume. There were three such pooled water samples per treatment utilizing water from all 12 replicate beakers. Each water sample was acidified to a pH of <2.0 with 1.0 ml of concentrated HNO_3.

To evaluate whether mercury and cadmium were quantitatively affecting

the food source and therefore introducing a nontreatment bias to the biological endpoints, an algal toxicity test was conducted with the green algae food source concurrently with the *Daphnia* exposure. All exposure conditions were identical to the *Daphnia* test except that there were three replicate beakers per metal concentration and no *Daphnia* were present in the beakers. At the end of 48 hr, algae were spun down in a centrifuge at 6089 g's for 10 min. Excess water was siphoned off, and algal pellets were resuspended in 10 ml of R.O. water. Samples were counted in a Neubauer counting chamber at 40X.

Treatment effects on mortality, growth, and reproduction were evaluated by one-way analysis of variance. The Waller-Duncan K-ratio t-test was used to separate means. Differences were considered statistically significant for $p < 0.05$.

Results

Survival

Mercury. *Daphnia* exposed to the highest mercury concentration (1.0 µg/ℓ) showed only 17-percent survival by the end of the 28-day experiment. Except for one *Daphnia* in the control, all those exposed to 0.0, 0.05, and 0.1 µg/ℓ survived the 28-day test (Figure 1). Survival of the *Daphnia* in the 0.5-µg/ℓ concentration at day 28 was intermediate (75 percent) to the other treatments.

Approximately half of the *Daphnia* that died in the 1.0-µg/ℓ treatment exhibited complete loss of setae and distal segments of the second antennae (locomotor appendages) approximately 3 to 5 days prior to death. The diminutive antennae could not maintain the *Daphnia* in the water column, its normal habitat. Affected organisms in the 1.0-µg/ℓ treatment propelled themselves along the bottom of the beaker in short jerky motions.

Cadmium. *Daphnia* exposed to the highest cadmium concentration (5.0 µg/ℓ) exhibited 100-percent mortality by day 21, while all those exposed to the three lowest concentrations (0.0, 0.1, 0.5 µg/ℓ) survived the 28-day experiment (Figure 2). Not surprisingly, survival of *Daphnia* in the remaining exposure concentration, 1.0 µg/ℓ, was intermediate to the other treatments.

Growth

Mercury. When expressed as mean lengths, growth was not significantly

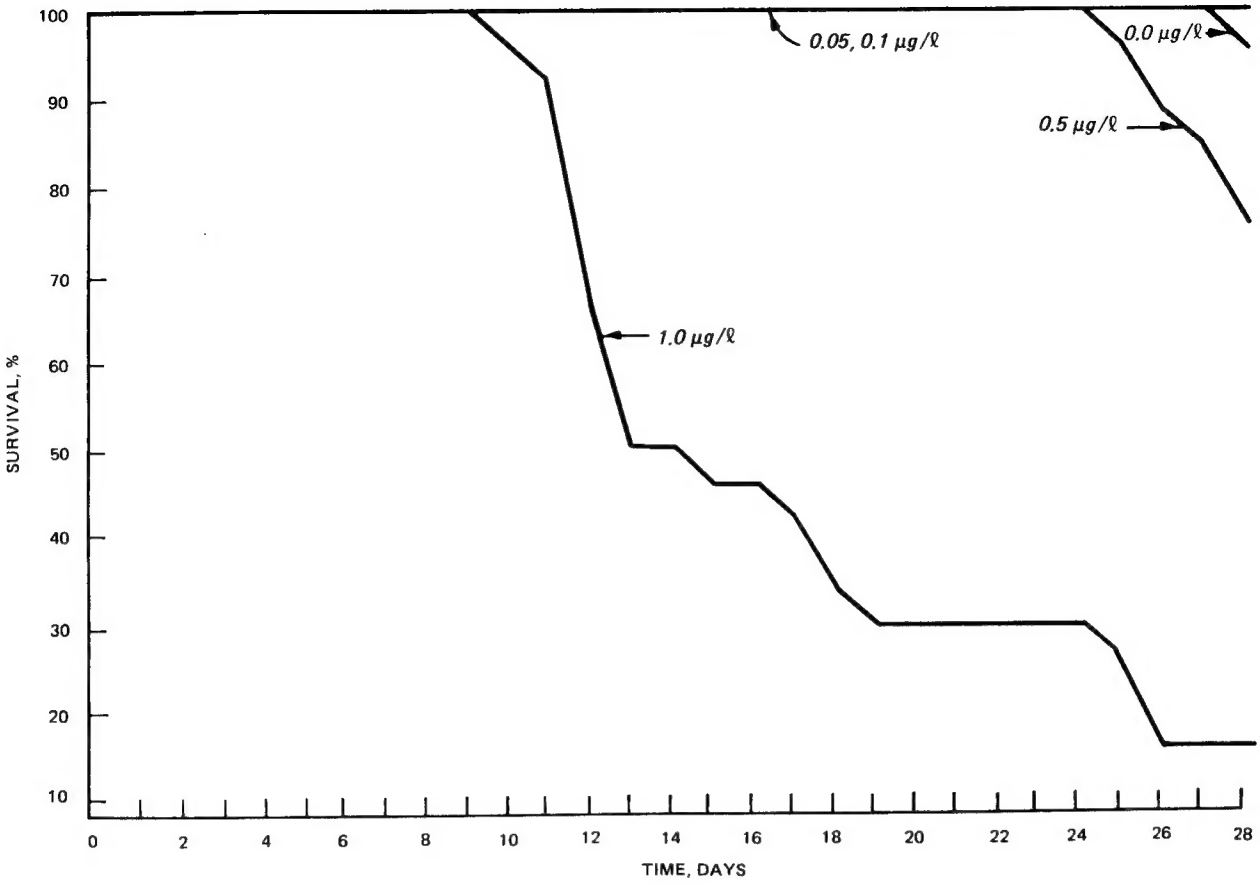

Figure 1. Survival of *Daphnia magna* during 28-day exposure to mercury

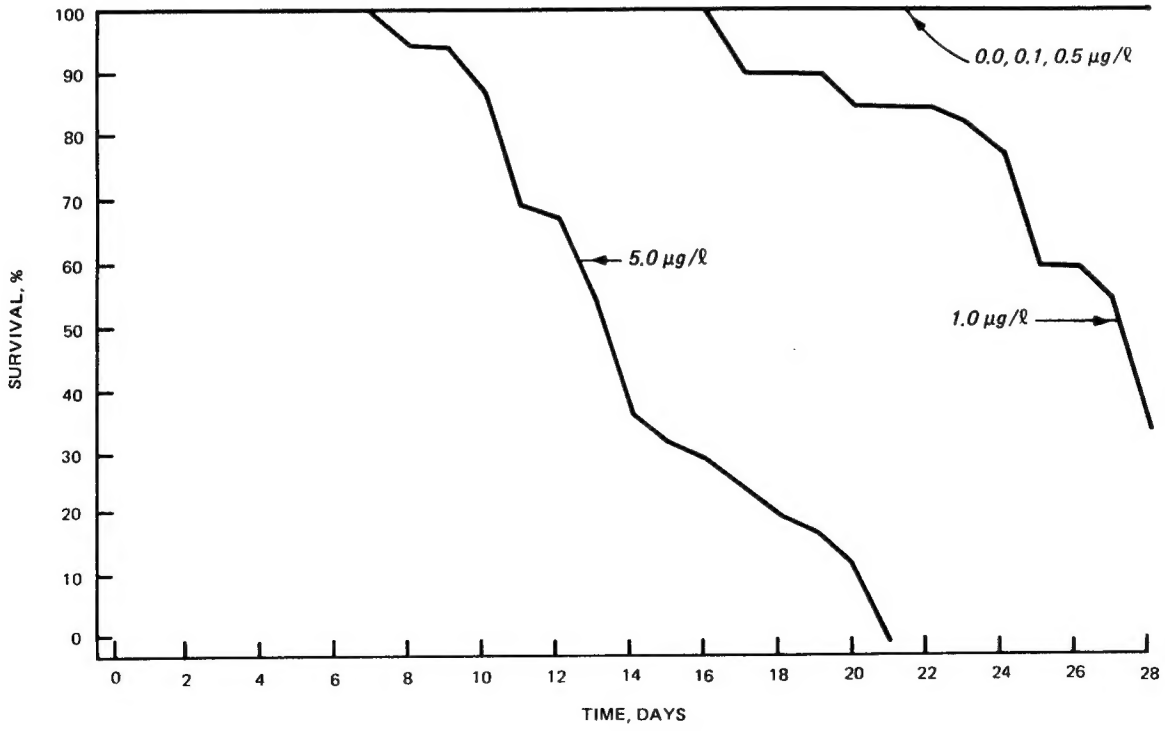

Figure 2. Survival of *Daphnia magna* during 28-day exposure to cadmium

affected by mercury exposure (Table 1). Mean lengths ranged from 5.12 to 5.33 mm. However, growth, expressed as dry weight, was significantly affected in an unexpected dose-related manner. The mean dry weight of *Daphnia* in the highest treatment (1.0 µg/ℓ) was 795 µg, which was significantly greater than the dry weights observed in the three lowest exposure concentrations (0.0, 0.05, 0.1 µg/ℓ). In those three groups, mean dry weights ranged from 511 to 558 µg. The mean dry weight of *Daphnia* exposed to 0.5 µg/ℓ was intermediate (610 µg) and significantly different from all other treatments.

Cadmium. Growth was significantly reduced in *Daphnia* exposed to 1.0 µg/ℓ cadmium compared to those in the three lower exposure concentrations (Table 2). Although growth data were not collected for *Daphnia* in the 5.0-µg/ℓ treatment due to high mortalities, daily observations indicated that these organisms were much smaller than those in the other treatments. Both measures of growth (mean lengths and dry weights) were significantly lower in the 1.0-µg/ℓ treatment (4.94 mm and 405 µg, respectively) compared to the three lower concentrations. In those three groups, mean lengths ranged from 5.20 to 5.26 mm and dry weights from 508 to 544 µg and were not significantly different from each other.

Reproduction

Mercury. There were no significant differences among mercury treatments for two measures of reproduction, i.e., time to first egg production or total neonates produced per female (Table 1). Total neonates produced per female ranged from 42 to 35. There was a significant depression in the total number of neonates produced per beaker in *Daphnia* exposed to the highest mercury concentration (1.0 µg/ℓ). This reduced production was due to mortality of the adults and not to a reduction in reproduction, per se.

Mean time to first appearance of eggs in the brood chamber ranged very narrowly from 5.9 to 6.0 days and was also not significantly affected by the mercury exposure (Figure 3). These similar mean values imply that egg production was extremely synchronous. This synchrony persisted throughout the experiment as evidenced by the appearance of successive broods (Figure 3). Six distinct broods were produced during the 28-day experiment with peaks in production occurring on days 9, 12, 16, 19, 23, and 26. Mercury does not appear to affect the timing of successive broods production.

Cadmium. The level of cadmium exposure affecting reproduction was similar to that observed for growth and survival. Again, there were no

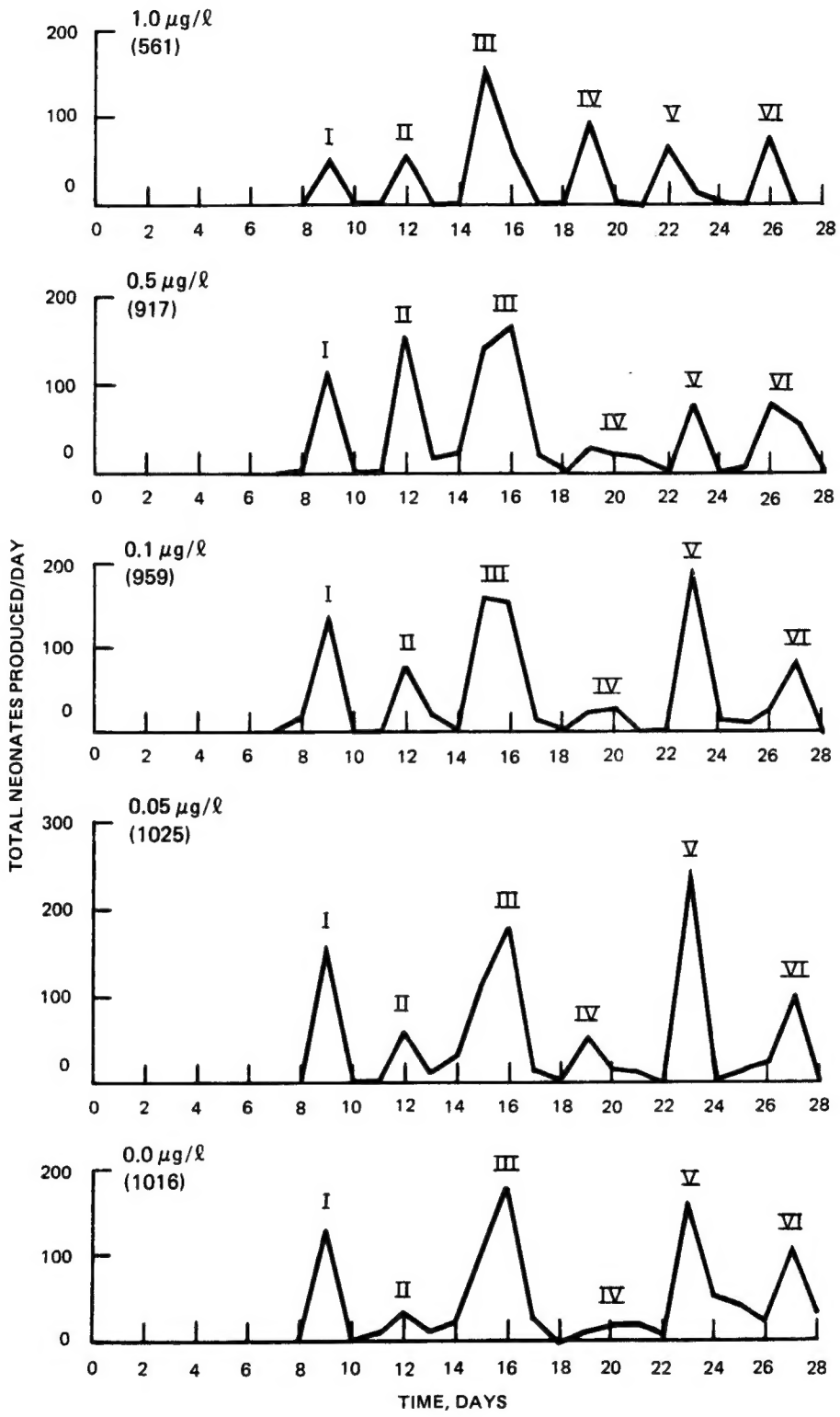

Figure 3. Daily neonate production of *Daphnia magna* during 28-day exposure to mercury. Values in parentheses represent the total production through 28 days. Roman numerals signify peaks in successive broods

significant differences among the 0.0-, 0.1-, and 0.5-µg/ℓ treatments for either measure of reproduction, i.e., time to first egg production or total neonates produced per female (Table 2). Reproduction was significantly depressed in *Daphnia* exposed to 1.0 and 5.0 µg/ℓ cadmium. Total neonates per female for *Daphnia* exposed to 1.0 and 5.0 µg/ℓ were 7.1 and 0.7, respectively. These values are significantly less than those for *Daphnia* in the lower exposure treatments (0.0, 0.1, and 0.5 µg/ℓ) in which mean values ranged from 39 to 42.

There was a significant depression in the total number of neonates produced per beaker exposed to 1.0 and 5.0 µg/ℓ cadmium. Time to first appearance of eggs in the brood chamber was also slightly but significantly delayed in *Daphnia* exposed to 1.0 and 5.0 µg/ℓ cadmium (6.4 days) compared to all other treatments (6.0 days) (Table 2). These statistically significant differences between very similar mean values are probably not biologically important but do imply that egg production was extremely synchronous. Indeed this synchrony persisted throughout the experiment as evidenced by the appearance of successive broods (Figure 4). Six distinct broods were produced during the 28-day experiment with peaks in brood production occurring on days 9, 12, 16, 20, 23, and 27. Cadmium does not appear to affect the timing of successive broods production.

Tissue residues

Mercury. Mean mercury tissue concentrations for *Daphnia* exposed to 0.0, 0.05, 0.1, 0.5, and 1.0 µg/ℓ, expressed on a dry-weight basis, were < 0.20, 0.69, 1.92, 5.47, and 5.47 µg/g, respectively. Due to high mortalities, only one sample consisting of four surviving *Daphnia* was available in the 1.0-µg/ℓ treatment at day 28. However, the concentration of mercury in this tissue sample along with that in the 0.5-µg/ℓ treatment were significantly greater than observed in the three lower treatments.

Cadmium. Measured water concentrations of cadmium in the exposure beakers were very similar to calculated concentrations (Table 3). Mean cadmium tissue concentrations for *Daphnia* exposed to 0.0, 0.1, and 0.5 µg/ℓ, expressed on a dry-weight basis, were 3.55, 4.49, and 6.58 µg/g, respectively. None of these values was significantly different from one another. Due to high mortalities, no tissue samples were collected from the 5.0-µg/ℓ treatment, and only a single sample consisting of the eight surviving *Daphnia* was available in the 1.0-µg/ℓ treatment at day 28. However, cadmium in this

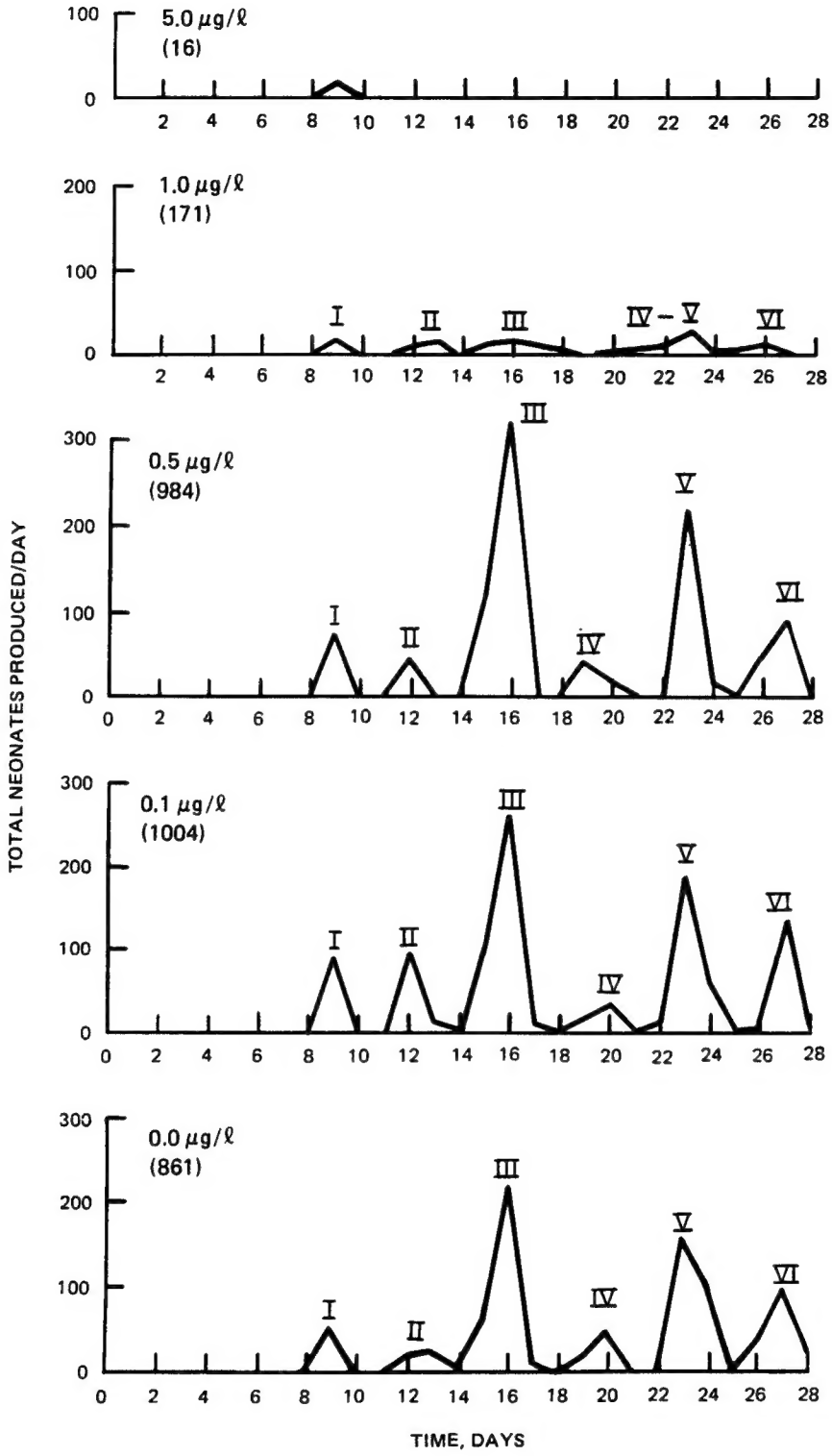

Figure 4. Daily neonate production of *Daphnia magna* during 28-day exposure to cadmium. Values in parentheses represent the total production through 28 days. Roman numerals signify peaks in successive broods

tissue sample appeared to be greater (11.8 µg/g) than found in the other three treatments.

Algal assay

Results of the algal toxicity test showed that there were no significant effects of either mercury or cadmium on the green algae used as the *Daphnia* food source.

Discussion

Mercury

Of the three biological effects examined (survival, growth, and reproduction), only survival was significantly affected in a detrimental manner when *Daphnia* were exposed to mercury for 28 days. Behavioral and morphological observations may help explain the unexpected dose-response pattern in dry weights. Since there was a very thin film of algae covering the bottom, the animals in the 1.0-µg/ℓ treatment were in direct contact with a spatially concentrated food source. The affected *Daphnia* were assumed to be feeding very well as evidenced by bright green digestive tracts and cleared feeding trails behind the *Daphnia* as they propelled themselves along the bottom of the beakers.

Some of the *Daphnia* exposed to 0.5 µg/ℓ were similarly affected, but the frequency of occurrence was greatly reduced compared to those in the 1.0-µg/ℓ treatment. Diminutive antennae were not observed in any *Daphnia* from the other (0.0, 0.05, and 0.1 µg/ℓ) treatments. It is speculated, therefore, that the observed pattern of dry weights was a combined consequence of reduced energetic costs associated with not having to maintain position in the water column coupled with increased caloric intake resulting from feeding on a spatially concentrated food source.

Cadmium

Results reported herein demonstrate a clear dose-response for cadmium-exposed *Daphnia magna*. Data for all the biological effects examined (survival, growth, and reproduction) indicated that *Daphnia* exposed for 28 days to 1.0 and 5.0 µg/ℓ cadmium were significantly affected in a detrimental manner, relative to *Daphnia* exposed to 0.0, 0.1, and 0.5 µg/ℓ. Similar results have been reported for the congener *Daphnia galeata mendotae*, in which growth and reproduction were impaired when exposed for 22 weeks to 4 µg/ℓ cadmium or

higher (Marshall 1978). Marshall reported mean tissue concentrations, expressed on a dry-weight basis, of <8.0, 17.6, 28.3, 42.8, and 51.9 µg/g ppm for *Daphnia* chronically exposed to 0, 1, 2, 4, and 8 µg/ℓ cadmium, respectively.

Conclusions

This study provided information about the sensitivity of *Daphnia magna* to mercury and cadmium. *Daphnia* with tissue levels less than 1.9 µg/g mercury or 6.6 µg/g cadmium were not adversely affected. However, *Daphnia* with tissue concentrations greater than or equal to 5.5 µg/g mercury or 11.8 µg/g cadmium exhibited diminished survival, growth, and reproduction.

Results suggest that a 28-day *Daphnia magna* toxicity test might be used in screening for water-column toxicity resulting from open-water disposal of a specific dredged material. The test may be used to predict safe and harmful levels of mercury and cadmium for *Daphnia magna* when survival, growth, and reproduction are measured.

Daphnia magna offers a short-term alternative test species with predictive values for the establishment of chronic-effects data for freshwater invertebrates. The relatively short life cycle of the species and the 28-day duration of the test, the small volume of water used in the tests, and the ease in handling and high fecundity of the organism make *Daphnia* an appealing alternative to the conduct of studies with organisms that require a longer term study that involves much greater volumes of water and complex laboratory equipment.

References

Buikema, A. L., Jr., Lee, D. R., and Cairns, J. 1976. "A Screening Bioassay Using *Daphnia pulex* for Refinery Wastes Discharged into Freshwater," *Journal of Testing and Evaluation*, JTEVA, 4(2):119-125.

Dunbar, A. M., Lazorchak, J. M., and Waller, W. T. 1983. "Acute and Chronic Toxicity of Sodium Selenate to *Daphnia magna* Straus," *Environmental Toxicology and Chemistry*, 2:239-244.

Marshall, J. S. 1978. "Population Dynamics of *Daphnia galeata mendotae* as Modified by Chronic Cadmium Straus," *Journal of Fishery Research Board of Canada*, 35:461-469.

Table 1. Survival, Growth, and Reproduction of *Daphnia magna* Exposed to Mercury for 28 Days under Static Renewal Conditions[a]

Nominal Exposure Concentration µg/ℓ	Proportion of Adults Surviving to Day 28	28-day Growth		Reproduction		
		Adult Length mm	Adult Dry Wt µg	Time to First Appearance of Eggs in Brood Chamber days	Total Neonates[b] per Female	Total Neonates[b] per Beaker
0.0	0.96 (0.04) n=12	5.24 (0.03) n=12	511 (10.0) n=23	6.0 (0.10) n=24	42 (1.9) n=12	85 (3.8) n=12
0.05	1.00 (0.00) n=12	5.21 (0.04) n=12	540 (13.0) n=24	6.0 (0.06) n=24	43 (1.5) n=12	85 (3.0) n=12
0.1	1.00 (0.00) n=12	5.12 (0.04) n=12	558 (10.7) n=24	5.9 (0.09) n=24	40 (1.1) n=12	80 (2.3) n=12
0.5	0.75** (0.10) n=12	5.21 (0.04) n=12	610* (42.0) n=17	5.9 (0.10) n=24	39 (2.6) n=12	76 (5.3) n=12
1.0	0.17** (0.09) n=12	5.33 (0.19) n=4	795** (122.8) n=4	5.9 (0.06) n=24	35 (9.5) n=12	47** (12) n=12

[a] Entries for each exposure concentration are consecutively mean value, (standard error), and n=number of replicates. Significantly different values are identified as follows:
* Significantly different from control treatment.
** Significantly different from all other treatment means ($p < 0.05$).

[b] Adjusted for daily records of mortality and assumes equal neonate production by each of two *Daphnia* per beaker.

Table 2. Survival, Growth, and Reproduction of *Daphnia magna* Exposed to Cadmium for 28 Days under Static Renewal Conditions[a]

Nominal Exposure Concentration µg/ℓ	Proportion of Adults Surviving to Day 28	28-day Growth		Reproduction		
		Adult Length mm	Adult Dry Wt µg	Time to First Appearance of Eggs in Brood Chamber days[b]	Total Neonates per Female[c]	Total Neonates per Beaker
0.0	1.00 (0.00) n=12	5.26 (0.06) n=12	508 (22.9) n=23	6.0 (0.0) n=23	39 (3.1) n=12	72 (2.4) n=12
0.1	1.00 (0.00) n=12	5.24 (0.05) n=12	514 (17.2) n=24	6.0 (0.0) n=2	42 (1.9) n=12	84* (3.8) n=12
0.5	1.00 (0.00) n=12	5.20 (0.03) n=12	544 (8.7) n=24	6.0 (0.0) n=24	41 (1.4) n=12	82 (2.7) n=12
1.0	0.33** (0.15) n=12	4.94* (0.06) n=8	405* (42.1) n=8	6.4* (0.2) n=22	7.1** (4.0) n=12	14** (7.9) n=12
5.0	0.00** (0.00) n=12	No Data	No Data	6.4* (0.1) n=14	0.7** (0.3) n=12	13** (0.7) n=12

[a] Entries for each exposure concentration are consecutively mean value, (standard error), and n=number of replicates. Significantly different values are identified as follows:
* Significantly different from control treatment.
** Significantly different from all other treatment means ($p < 0.05$).

[b] Includes only those Daphnia for which any egg production was observed.

[c] Adjusted for daily records of mortality; assumes equal neonate production by each of two *Daphnia* per beaker.

Table 3. Mercury and Cadmium in Tissue and Water Samples[a]

Nominal Water Concentrations µg/ℓ	Measured Water Concentrations µg/ℓ	*Daphnia* Tissue Concentrations µg/g
Mercury:		
0.0 0.023 (0.02) n=3	0.2 (1.63) n=3	
0.05 (0.14) n=3	0.06 (0.02) n=3	0.69
0.1 0.06 (0.03) n=3	1.92* (0.74) n=3	
0.5 0.25 (0.02) n=3	5.47** (0.22) n=2	
1.0 0.73 (0.09) n=3	5.47** (0.0) n=1	
Cadmium:		
0.0 <0.10 n=3	3.55 (1.63) n=3	
0.1 0.10 (0.0) n=3	4.49 (0.59) n=3	
0.5 0.50 (0.0) n=3	6.58 (0.91) n=3	
1.0 0.97 (0.09) n=3	11.8**[b] n=1	
5.0 4.5 (0.53) n=3	No data[b]	

[a] Entries for each exposure concentration are consecutively mean value, (standard error), and n=number of replicates. Significantly different values are identified as follows:
*Significantly different from control treatment,
**Significantly different from all other treatment means ($p < 0.05$).

[b] Insufficient tissue for replicate samples due to low percent survival at day 28.